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Deformulation of a solid pharmaceutical form using computed tomography and X-ray fluorescence

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Abstract. Deformulation of medicines is of undeniable importance, since it can be utilized both to unravel the chemical composition of the excipients integrating a pharmaceutical formulation of a specific medicine and as an important tool to conduct morphometric studies of the formulation under study. Such strategy may be utilized in analytical studies aiming at quantifying the components of reference drugs, or in the identification of putative counterfeit pharmaceuticals. Deformulation makes use of physicochemical analysis tools to characterize, from the chemical point of view, the components integrating medicine pharmaceutical formulations and from the physical point of view, the morphological part of the pharmaceutical formulation. The techniques of computer tomography (SkyScan 1174 - Bruker microCT) and X-ray fluorescence analyses (using an X-ray source with W-anode from Hammatsu Photonics and Silicon Drift detector from Amptek) were successfully used in performing a process of deformulation of a solid pharmaceutical formulation of tablets, utilized herein as a model medicine for controlled drug release. The analytical methods used in this work, proved their effectiveness for the main goal of this study, which aimed to characterize a pharmaceutical formulation via its deconstruction.

1. Introduction

Deformulation has been increasingly practiced by pharmaceutical companies whose primary production focus is the manufacture of medicines marketed under the name of Generic. A Generic medicine is one that contains the same drug (active ingredient) in the same dose and dosage form, is administered via the same route and with the same therapeutic indication, and shall present the same biological safety as the reference drug in the country. To be interchangeable with the reference drug, the Generic product must pass and be approved in bioequivalence testing [1]. To achieve both the same therapeutic performance and safety of (so-called Reference) medicines, pharmacotechnical and technological development is required, together with significant financial investment and skilled labor. To facilitate copying into a Generic (a simpler and less expensive in investment), deformulation of the reference medicine is practiced in order to acquire knowledge about the chemical composition of the

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compounds present in these medicines and to know the physical characteristics of the formulation, such as the mechanism of drug release, the thickness of the coating, the porosity, the morphological features, the process of drug release, etc. Deformulation enables both the decomposition of the medicine to copy and the identification of counterfeit medicines. Non-destructive analysis techniques such as computed tomography via X-ray transmission (CT) and X-ray fluorescence (XRF) are useful for deconstruction, because the former can provide the physical characteristics whereas the second provides information on the elemental constitution of the formulation, i.e. chemical composition. The results of the analyses are easily comparable and may be confronted in case of rebuttal. CT began to be used back in 2003 in the pharmaceutical field as a new tool for the nondestructive analysis of solid dosage forms, by Farber et al. [2] and Ozeki et al. [3]. The great advantage in the use of CT when compared to more traditional techniques, lies in the fact that it can be used to view not only the surface, but also the inside of objects with varying dimensions, shapes and densities, without any loss of sample. Additionally, CT enables the morphological characterization of regions inaccessible to other analytical equipments. By using CT, it is possible to determine the coating thickness of a tablet or even analyze the several layers of tablet materials subjected processing via multiple compression. Traini et al. [4] emphasize that the main analytical methods used to characterize the interior of solid dosage forms are the use of techniques of mercury porosimetry and gas adsorption. However, these researchers make it clear that, if the purpose is to conduct dynamic studies, i.e. to track changes in the internal structure of the tablets following changes in porosity during the disintegration process, the aforementioned porosimetry techniques cannot be used since they are destructive techniques preventing the use of the sample in more than one type of analysis, and recommend in these cases the use of CT. Li et al. [5] propose the use of the CT technique to study processes of drug release, using three-dimensional tomographic imaging with X-rays from synchrotron light. Oliveira Jr. et al. [6] showed, in a recent study, that it is possible to use the computed tomography technique for studies of disintegration of solid dosage forms. Scanning electron microscopy (SEM), X-ray diffraction analysis (XRD), X-ray attenuation and CT scans are analytical techniques indicated for morphological studies [7, 8, 9], whereas atomic force microscopy has been used in the analysis of the effects produced by mechanical processing upon the surface of pharmaceutical powders [10]. X-ray fluorescence analysis (XRF) [11] has been used not only to display but also to quantify the presence of trace elements in pharmaceutical formulations [12]. Computed tomography via X-ray transmission (CT) and X-ray fluorescence analysis (XRF) were utilized to perform deformulation of a solid pharmaceutical form. As a model for the study undertaken, one used a medicine in the form of coated tablets, the purpose of which is the controlled release of the drug through the increase in the osmotic pressure of the system following water absorption - Osmotic Release Oral System (OROS™). There is currently a great interest within the pharmaceutical industry in the production of these tablets, since due to expiration of the protection periods of their patents there is no longer the need to perform new clinical trials, and thus this type of medicines may be marketed immediately.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals.

As model medicine, one utilized Adalat Oros™ tablets (30 mg nifedipine, Lot MS-1.0429.0001.137-9, Bayer AG, Leverkusen, NRW, Germany), acquired in a local Brazilian drug store.

2.1.2. Analytical equipment.

A computed tomographer via X-ray transmission from Bruker microCT (model SkyScan 1174, Kontich, Belgium), and an X-ray fluorescence spectrometer (XRF) from Amptek Inc. (Bedford MA, U.S.A.) were utilized in all non-destructive analyses. The analysis software utilized for processing the

tomographic images were CTVox™ (version 2.6.0 r908-64bit, from Bruker microCT) and CTan™ (version 1.13.5.1-64bit, from Bruker microCT).

2.2. Experimental procedures

In this work, one utilized either dry tablets of Adalat Oros™ or tablets maintained submerged in ultrapure water during predetermined intervals of time. The tomographic images were gathered under two different conditions: (i) dry tablets, and (ii) tablets submerged in ultrapure water for a period of 1225 and 1440 min, followed by an air drying period of 48 h in a room free from dust at room temperature and in the dark. The tablets were then placed in the interior of the tomograph chamber so as to produce the tomographic images. The tomographic images were obtained using the following configurations of the tomographic system: operating voltage set at 35 kV and electric current of 800 μA. The technique employed for obtaining a tomographic image involved the acquisition of a large number of radiographs of the object, obtained by measuring the intensity of X-rays transmitted through the sample, at different angular positions. The angular step utilized was 1°, the samples were rotated 180 degrees, producing 180 radiographs (projections) per image, each containing 652×652 pixels with spatial resolution of 19.52 μm. At the outlet of the X-ray source one utilized a filter of Al with 0.25 mm thickness. Appropriate mathematical algorithms were then used to reconstruct the three-dimensional tomographic images (3D) of the object, through the appropriate composition of bi-dimensional images (2D). In the present research work, the three-dimensional images possessed 652×652×652 pixels and the same spatial resolution of the 2D images, and thus the volume of data generated for each tablet is isotropic with relation to the spatial resolution. In addition, a tomographic image of a dry tablet with a better spatial resolution was also produced, using the following experimental configurations: operating voltage set at 50 kV and electric current of 800 μA, Al filter with 0.5 mm thickness at the outlet of the X-ray source, 180° spatial rotation of the object with angular increments of 0.7°, thus generating three-dimensional images with 984×984×984 pixels and with a spatial resolution of 9.76 μm. Having all the projections (radiographs gathered at each angular position), one utilized the software NRecon™ from Bruker (version 1.6.9.4, Kontich, Belgium), which uses the algorithm of Feldkamp et al. [13] in the process of reconstruction of the tomographic images. The X-ray fluorescence equipment utilized consisted in a compact system from Amptek Inc. (Bedford MA, U.S.A.) and was composed by a silicon detector (Silicon Drift Detector) with an area of 25 mm^2 and 500 μm thickness, protected by a Beryllium window of 12.5 μm. The X-ray source utilized in excitation of samples is equipment manufactured by Hamamatsu Photonics Company, model L6731-01, that uses a target of tungsten and operates with variable voltages from 20 kV to 80 kV and maximum electric current of 100 μA. To acquire the X-ray fluorescence data one utilized the software DppMCA™ from Amptek Inc. (version 1.0.0.12, Bedford MA, U.S.A.) whereas the data gathered was analysed using the software XRF-FP™ also from Amptek Inc. (version 5.2.9). The process of excitation of samples was carried out using an Al collimator with 2-mm orifice opening at the outlet of the X-ray source. The X-ray source was configured to operate at a voltage of 25 kV and electric current of 99 μA. Due to the fact that each chemical element possesses a unique set of atomic energy levels, each chemical element also emits a unique set of characteristic X-rays when properly excited, that are characteristic of the element that emitted them. Additionally, the intensity of a line of characteristic X-rays depends on the number of corresponding atoms that have been excited in the sample. The fluorescence technique used in this study was X-ray Fluorescence by Energy Dispersion (EDXRF), also known as non-dispersive X-ray fluorescence. In this technique, the characteristic X-rays are selected and quantified by using a detection system which operates with detectors able to discriminate between X-rays of close energies. The technique of X-ray fluorescence requires a minimal sample preparation, and the measurements performed in this study were conducted by setting to 900 s the time of sample excitation. The X-ray fluorescence analyses were carried out both at the surface and interior of dry Adalat Oros™ tablets. The fluorescence system was calibrated using a reference standard sample supplied by the "National Institute of Standards and Technology – NIST" (Stainless Steel SS316), containing the chemical elements Cr, Mn, Fe, Ni, Cu and Mo at known

concentrations and using samples especially prepared for this purpose, containing known concentrations of the following elements: Mg, P, Cl, K, Ca and Ti. Quali-quantitative analysis of fluorescence data was performed using the software XRF-FP™ from Amptek Inc.

3. Results and discussion

In the process of deformulation of a pharmaceutical form, the greater the detail of the physicochemical characteristics of the dosage form being studied, the easier one can reproduce it. Figure 1 shows a typical tomographic image of a tablet of Adalat Oros™. In figure 1A one can see the outside of the pharmaceutical form, in figure 1B the interior of a dried tablet can be seen after a digital cut of the image and in figures 1C and 1D the interior of the tablets after a digital cut of the images gathered following submersion of the tablets in ultrapure water for a period of 1225 and 1440 min, respectively.

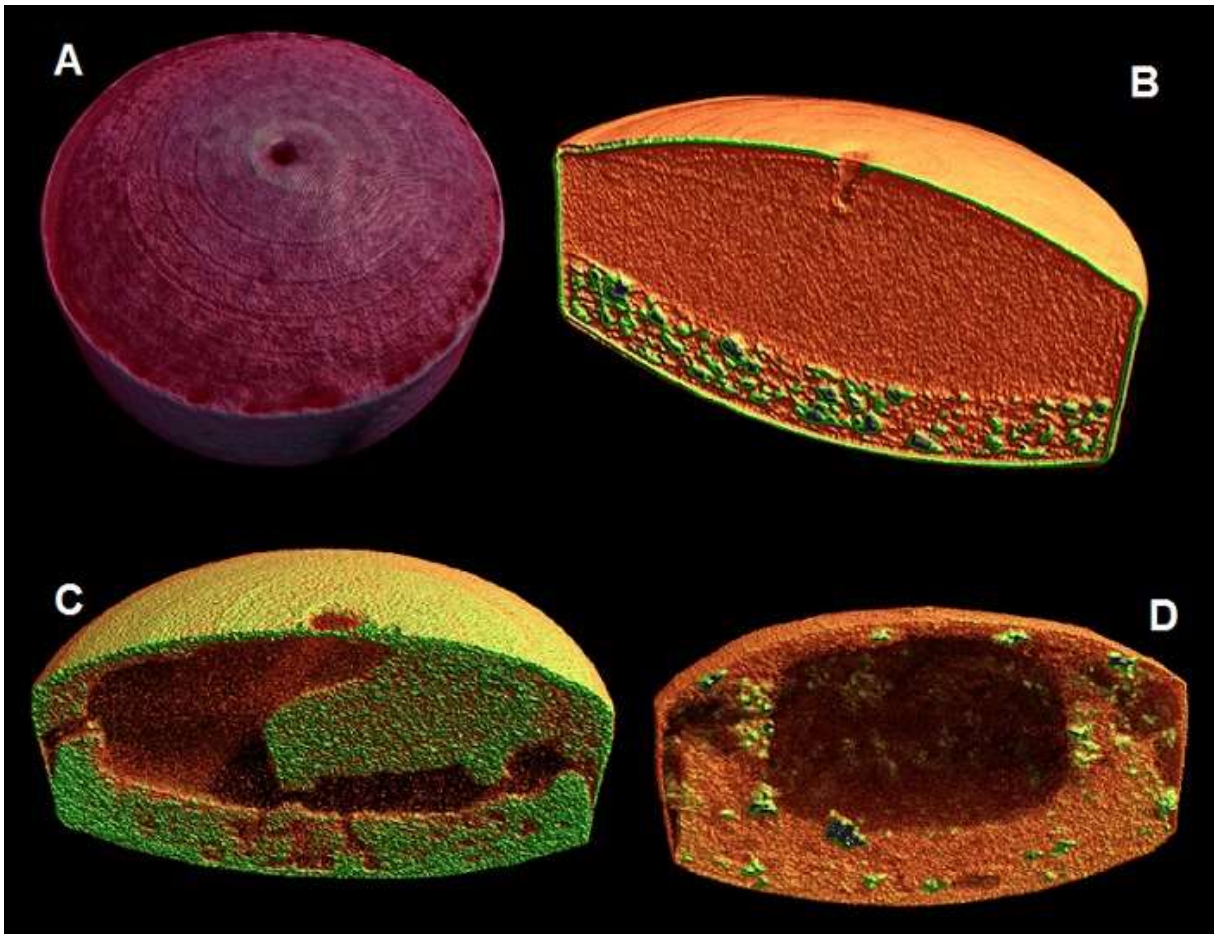

Figure 1. External part of the intact tablet of Adalat Oros™ (A), sight of its interior showing the drug (upper part) and the osmotic agent (lower part) (B), tablet of Adalat Oros™ after 1225 min (C) and 1440 min (D) of submersion in ultrapure water followed by static air drying at room temperature during 48 h prior to gathering the tomographic images.

The images in figure 1 show important details for the deformulation process of the pharmaceutical form. Through the analysis of the images in figures 1A and 1B one can obtain of morphological information of the dosage form, such as the size of the orifice through which the drug is expelled, the dimensions of the surface polymer that coats the tablet, the density and/or porosity of the drug packaged in the core of the dried pharmaceutical form, the thickness of the layer of sodium chloride crystals working as osmotic agent (osmotic pump), etc. Tomographic images like the ones displayed in

figures 1C and 1D can be used for dynamic studies on the process of tablet disintegration. By calculating the initial volume of drug present in the interior of the dosage form and comparing with the volumes after each period of time under submersion in ultrapure water, it is possible to determine the rate of disintegration of the tablet. All these morphological information are susceptible to be extracted from a CT image, since materials having different densities absorb different amounts of radiation, and thus appear in the tomographic images with different shades or colours, depending on whether the images were reconstructed in shades of a single colour (8-bit) or using several colours (16-bit or 24-bit). The morphological information gathered from the dosage form under study is displayed in Table 1.

Table 1. Morphologic characteristics of a dry tablet of Adalat Oros™. The data is presented as the average of 10 measurements together with their respective standard deviations.

Morphologic characteristics	Average value	Standard deviation
Width (mm)	9.13	0.03
Height (mm)	5.3	0.5
Orifice diameter (mm)	0.48	0.04
Thickness of the coating layer (mm)	0.095	0.005
Thickness of the layer of sodium chloride crystals (mm)	1.34	0.02
Porosity of the nifedipine drug (%)	9.1	2.4
Total volume of the tablet (mm^3)	258	6
Volume of the nifedipine drug (mm^3)	164	8

Another important issue regarding deformulation is the knowledge of the elemental constituents present in the original formulation, the drug and excipients. There are many techniques of elementary analysis, but in most cases one single technique is not able to identify and/or quantify all the elemental present in the formulation, thus requiring the use of more than one analytical technique, since some of these are sensitive to organic elements, others to the presence of inorganic elements, some are destructive, some do not possess the required accuracy, and so on. In this context, we discuss the use of the technique of X-ray fluorescence (XRF) in the analysis of medicines, due to the fact that its use is relatively recent in the pharmaceutical industry, by being non-destructive, not requiring specialized technical personnel to use it, because of its relatively low cost and because it does not require any previous sample preparation. XRF, with the detectors and X-ray sources currently available on the market, can be used to quantify chemical elements present in a formulation, as long as they have an atomic number higher than that of the chemical element Magnesium (Z=12) through chemical element Fermium (Z=100), with an accuracy of approximately a few parts per million (ppm). If the fluorescence system is contained within an apparatus able to allow vacuum, it is possible to perform elemental analysis departing from the chemical element Boron (Z=5). The X-ray fluorescence analyses of tablets of Adalat Oros™ were made in both the external and internal parts of the tablets. In the inner part of the medicine, the analysis was conducted in two distinct regions, the region that concentrates the elements responsible for the osmotic pump and the region where the drug nifedipine resides. Figure 2A shows the results obtained in the elemental analysis of the external part of Adalat Oros™ tablets, with the following main chemical elements being identified: titanium ($E_{k\alpha}$ = 4.51 keV and $E_{k\beta}$ = 4.93 keV), and iron ($E_{k\alpha}$ = 6.40 keV and $E_{k\beta}$ = 7.06 keV).

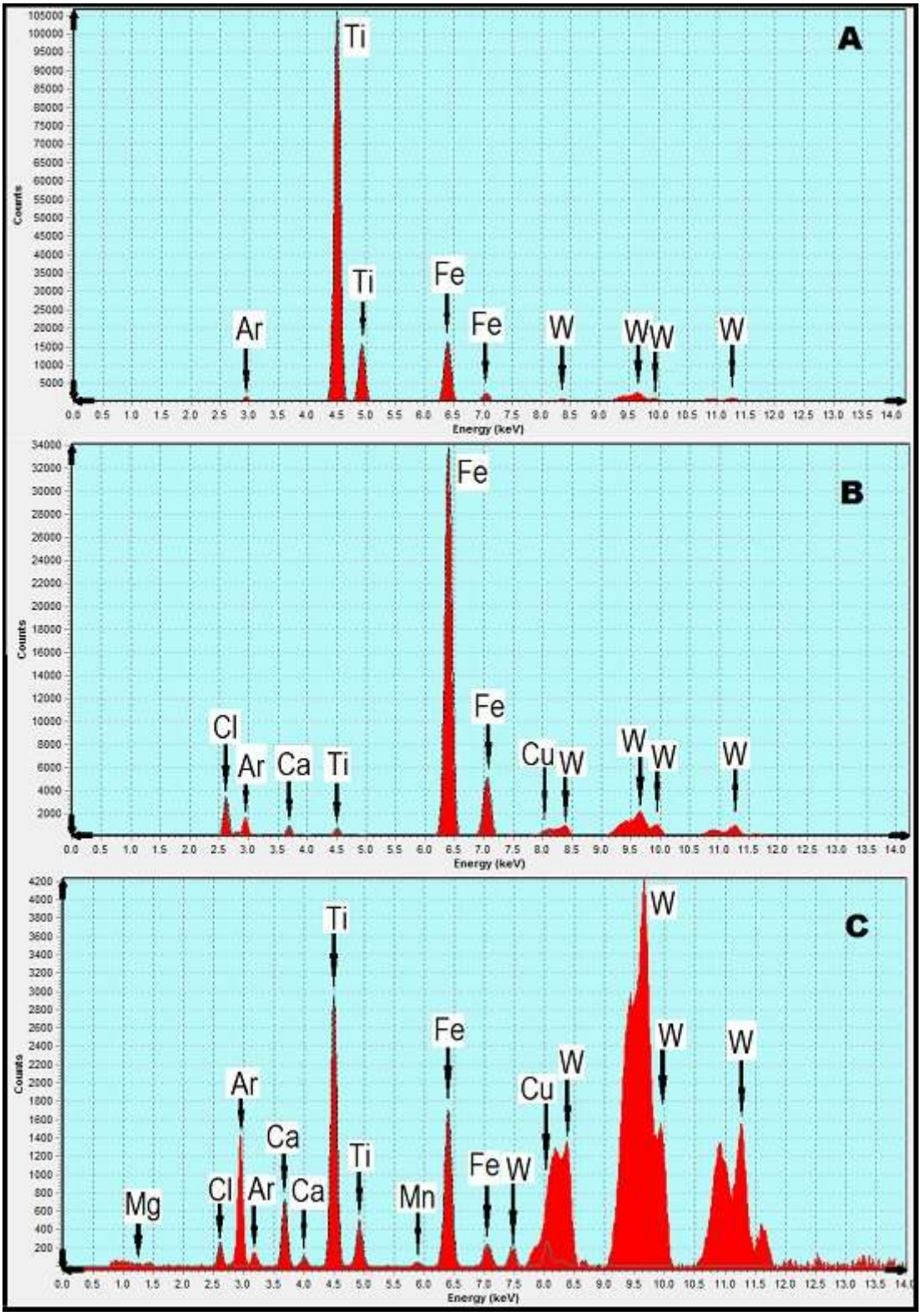

Figure 2. X-ray fluorescence spectra of the external part (A) and of the inner part of a tablet of Adalat Oros™, i.e. the region of osmotic pump (B) and the region that contains the drug nifedipine (C).

The element tungsten ($E_{L\alpha}$ = 8.40 keV, $E_{L\beta}$ = 9.67 keV, among other lines of tungsten) appears due to the target used in the X-ray source and the element argon ($E_{k\alpha}$ = 2.96 keV) appears due to its presence in the atmospheric air. Figure 2B shows the chemical elements identified in the inner part of the tablet of Adalat Oros™, of the side of the tablet where is located the osmotic pump. The main elements found in this region were: chlorine ($E_{k\alpha}$ = 2.62 keV), calcium ($E_{k\alpha}$ = 3.69 keV), titanium ($E_{k\alpha}$ = 4.51 keV), iron ($E_{k\alpha}$ = 6.40 keV and $E_{k\beta}$ = 7.06 keV) and copper ($E_{k\alpha}$ = 8.04 keV) in addition to elements argon ($E_{k\alpha}$ = 2.96 keV) and tungsten ($E_{L\alpha}$ = 8.40 keV, $E_{L\beta}$ = 9.67 keV), among other lines of tungsten). Figure 2C shows the chemical elements identified in the inner part of the tablet of Adalat Oros™, in the region that contains the drug nifedipine. The main elements found in this region were: magnesium ($E_{k\alpha}$ = 1.25 keV), chlorine ($E_{k\alpha}$ = 2.62 keV), calcium ($E_{k\alpha}$ = 3.69 keV), titanium ($E_{k\alpha}$ = 4.51 keV), manganese ($E_{k\alpha}$ = 5.89 keV), iron ($E_{k\alpha}$ = 6.40 keV and $E_{k\beta}$ = 7.06 keV) and copper ($E_{k\alpha}$ = 8.04 keV) in addition to element argon ($E_{k\alpha}$ = 2.96 keV) and tungsten ($E_{L\alpha}$ = 8.40 keV, $E_{L\beta}$ = 9.67 keV), among other lines of tungsten). Table 2 displays the amounts of each chemical element found both in the coating (external) and in the inner parts of the tablets, as percentages of the total tablet mass. According to the pharmaceutical manufacturer of Adalat Oros™ tablets, the medicine contains in addition to nifedipine the following inert components: hydroxypropyl methylcellulose (HPMC), magnesium stearate, polyethylene oxide, sodium chloride, red iron oxide, cellulose acetate, polyethylene glycol, hydroxypropyl cellulose, propylene glycol and titanium dioxide. The technique of X-ray fluorescence analysis used in this study cannot separate and quantify constituents having atomic numbers below that of magnesium element. The chemical formula of nifedipine is $C_{17}H_{18}N_2O_6$ and, since all the elements present in the molecule have an atomic number lower than Z=12, we cannot quantify them. Regarding the chemical element sodium, by having atomic number Z=11, its characteristic X-rays cannot reach the detector, and thus they do not appear in the spectra of figure 2. The chemical elements calcium, manganese and copper are contaminants, since they do not appear in the patient information leaflet provided by the pharmaceutical manufacturer. The elements iron and titanium must have originated from the red iron oxide and the titanium dioxide used in the tablet coating process. Regarding the remaining contaminants, we cannot infer about their origin, but they are probably linked to the production process. The element chlorine is present in the sodium chloride used as osmotic agent, which is the responsible for the increase in pressure inside the pharmaceutical form and concomitant expulsion of the drug (osmotic pump). The element magnesium is one of the excipients indicated by the manufacturer used in formulating the medicine. In this work, we clearly demonstrated that XRF can be used as a powerful tool for elemental analysis, and that it can be used both in qualitative identification and quantification of the elements present in a pharmaceutical formulation. It is possible to perform a quantitative analysis of light elements (Z<12), but in this case there is the need to place the experimental setup (sample, X-ray source and detector) within an evacuated chamber. The major focus of the research work undertaken was to demonstrate the potential of using the techniques of computed tomography and X-ray fluorescence analysis to perform deformulation of medicines.

4. Conclusion

By using CT images, it was possible to make a detailed physical analysis of a pharmaceutical form, including dynamic studies of the drug release process. All the morphological characteristics of the tablets were studied using three-dimensional tomographic images. The region of the tablet housing the drug, the layer used as osmotic agent and the coating characteristics including the orifice used to equilibrate the osmotic pressure and release the drug, were clearly identified. One also demonstrated the possibility of using CT for dynamic studies, by measuring the porosity. The technique of X-ray fluorescence analysis complemented the information gathered from the tomographic images, by providing identification of the elemental constituents present in the dosage form both in the coating and in the tablet matrix. It was demonstrated, how the techniques of computed tomography and X-ray fluorescence analysis can be used together to perform deformulation of a modified release dosage form.

Table 2. Concentration of the chemical elements found in the external and inner parts of tablets of Adalat Oros™, expressed in percentage of the total tablet mass.

Part of the Adalat Oros™ tablet	Chemical element	% (w/w)
Exterior – coating	Titanium	33.33 ± 0.09
	Iron	3.65 ± 0.02
Interior - side of the osmotic pump	Chlorine	10.3 ± 0.2
	Calcium	1.50 ± 0.05
	Titanium	0.54 ± 0.02
	Iron	3.80 ± 0.02
	Copper	0.024 ± 0.001
Interior - side of the drug nifedipine	Magnesium	6.4 ± 3
	Chlorine	1.5 ± 0.1
	Calcium	0.80 ± 0.03
	Titanium	1.54 ± 0.02
	Manganese	0.005 ± 0.001
	Iron	0.16 ± 0.003
	Copper	0.005 ± 0.0009

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